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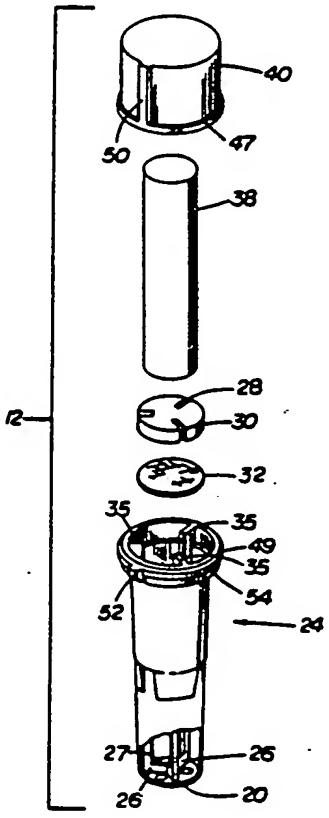
(54) Title: DUAL ABSORBENT ANALYTE DETECTION

(57) Abstract

A test device (12) and method for detecting an analyte in a liquid sample by treating the sample with liquid reagents to form a detectable product. The device (12) includes a reaction zone (32) capable of retaining the detectable product and a control absorbent (30) in liquid-transferring contact with the reaction zone (32). The control absorbent (30) has a predetermined liquid-absorbing capacity and is positioned such that when the control absorbent (30) is filled to capacity, it effectively meters a predetermined flow through the reaction zone (32). The device also includes (with or without the control absorbent (30)): an absorbent reservoir (38); means for retaining the absorbent reservoir (38) in a first position spaced apart from liquid-transferring contact with the control absorbent (30); and means for moving the absorbent reservoir (38) from the first position to a second position in liquid transferring contact with the control absorbent (30).



DUAL ABSORBENT ANALYTE DETECTION

Background of the Invention

This invention relates to devices and methods for detecting an analyte, for example, using an immunoassay.

An analyte in a sample may be detected by treating the sample with various reagents, such as labeled immunological binding partners to the analyte and reagents to enable detection of the label. Often, the sample must be washed between administration of various reagents.

An accurate assay often depends on controlling the amount of reactants exposed to the sample and the duration of the reactions taking place. It is desirable to control these variables in a way that enables use of the assay kit by individuals of widely varying skill and experience, so that variations in individual technique do not materially alter the result. Consistency and accuracy can be difficult when a color-generating indicator must be evaluated, particularly if any background color is generated. Test reagents should be tested to verify their activity, which may erode over time. Also, it is desirable to reduce the time necessary to perform the assay. It is desirable to have the ability to assay extremely small sample volumes with relative low concentrations of analyte, and/or to detect relatively small differentials in analyte concentrations. Finally, it is desirable to assay whole blood samples without complex centrifuging equipment.

One method for adding and washing reagents in an immunoassay uses an absorbent material to move liquid washes and reagents through a solid substrate (such as a membrane) to which other reactants are immobilized.

Cole et al. U.S. 4,246,339, discloses an immunoassay test device including sorbent material 18 for drawing liquid through a microporous membrane 22 at the bottom of a test well 20. The sorbent material is resiliently biased away from the membrane, and it draws liquid through the membrane only when the two are forced together to overcome the bias. Sorbent material 18 comprises a surface layer 36 which is hydrophobic and a bulk portion 38 which is wettable. Reagents are added serially to the test well and, after each reagent has been in the well for a prescribed time, the membrane and sorbent material are forced together to draw off liquid before the next reagent is added.

Tom U.S. 4,366,241, discloses an immunoassay device having two bibulous zones, an analyte binding partner being non-diffusively fixed in the first zone (the "immunoabsorbing zone"), and the second layer being a reservior zone, either directly or indirectly in liquid-receiving relationship with the first zone to pull liquid through and out of the first zone. The immunoabsorbing zone is a portion of a flat test strip which is exposed by a hole in a protective coating.

Hossom U.S. 4,623,461, discloses a test device having a filter which feeds a specimen to a flat absorbent material having a reaction zone surrounded by peripheral zone. An annular ring 40 of absorbent material is positioned around the peripheral zone, so that fluid is drawn radially outward from the reaction zone, through the peripheral zone, and into the absorbent material.

Bagshaine U.S. 3,888,629, discloses an assay device having a matrix pad 17 which may be pre-treated with one binding partner. Absorbent material 23 is forced into intimate contact with matrix pad 17 to increase the speed of filtration through the matrix pad.

Kondo U.S. Pat. 4,270,920, discloses multiple reagent layers arranged on a single horizontal support. A porous spreading layer (40 in Fig. 1 or 41 and 44 in Fig. 3) spreads the sample as it moves into the reagent layers, so that a relatively small sample volume will be spread evenly over the reagent layers.

Lawrence U.S. Pat. 4,365,970, Oksman U.S. Pat. 4,578,358, Brewer U.S. Pat. 4,486,540, and Guandagno U.S. Pat. 4,541,987, disclose test devices comprising positive and negative control spots on a test pad or slide.

Applicants' assignee, Agritech Systems, Inc., has marketed an immunoassay device comprising positive and negative control spots. Separation of the spots is maintained by spotting the reagents a sufficient distance from each other.

Various companies (e.g. Syntex's AccuLevel and Hybritech's ICON) have marketed self-contained immunoassays using a membrane as a solid support. See Weiss and Blankstein, American Clinical Products Review, May/June (1987). Latex particles, to which ligands have been attached, have been used in agglutination assays. Bangs, American Clinical Products Review, May/June (1987).

Summary of the Invention

One aspect of the invention generally features a test device having a control absorbent in liquid-transferring contact with an aqueous permeable, aqueous insoluble reaction zone, adapted to retain the detectable reaction product formed when analyte in the sample is treated with at least one liquid reagent. The control absorbent has a predetermined, limited, standard, liquid-absorbing capacity, and it is adapted to absorb a predetermined volume of liquid from the

sample after the sample passes through the reaction zone. That predetermined, limited, standard volume is selected to enable reliable detection of the analyte, taking into account the reagent volumes and concentrations present, the mode of detecting the reaction product, and the analyte concentration level at which discrimination is desired. The control absorbent and the means defining a reaction zone are positioned in the device in the manner that, by filling the control absorbent to capacity, it effectively meters a predetermined limited flow between a first region of the reaction zone, which is exposed to receive liquid sample, and a second region thereof, spaced from the first region, which is in liquid transferring relation with the control absorbent.

A second aspect of the invention generally features a device that includes: an absorbent reservoir; means retaining the absorbent reservoir in a first position spaced apart from liquid-transferring contact with the control absorbent or the reaction zone; and means moving the absorbent reservoir from the first position to a second position, in which the reservoir is in liquid-transferring contact with the control absorbent and/or the reaction zone.

The two aspects can be combined in a single device so that the control absorbent draws the predetermined sample volume into the reaction zone while the absorbent reservoir is in the first position; then the absorbent reservoir is moved into the second position, and the means defining a reaction zone is contacted with at least one of the liquid reagents which is drawn through the reaction zone into the absorbent reservoir.

Preferred embodiments of the invention include the following features. In the first position the absorbent reservoir is retained above the reaction zone, and it is moved from the first position to the second position, where it rests and is retained in liquid transferring contact above the reaction zone. A plurality of liquid reagents can be drawn upwardly through the reaction zone into the absorbent reservoir without additional reservoir movement. A latch means can be used to restrain movement of the absorbent reservoir from the second position back to the first position, so a plurality of liquid reagents can be introduced to the reaction zone while the absorbent reservoir is latched in the second position. The device also can include a means to release the latch after the detectable product is formed, so the absorbent reservoir is returned to the first position while the reaction product is being detected. At least one reactant participating in a reaction to form the detectable product is included in the reaction zone; for example, a specific binding partner for the analyte can be included in the reaction zone to trap the analyte there. The reaction zone is impermeable to the analyte. The reaction zone is defined on a flat member, and the two regions of the zone are opposite faces of the member The reaction product may be detected by a characteristic of the product, such as color intensity, optical density, reflectance density, pH, fluorescence, or conductivity. For example, it may be detected by external visual inspection of the means defining a reaction zone. A contrast region surrounding said reaction zone aids detection by contrasting with the reaction zone in respect to the characteristic detected. An intensity scale may be included to aid

quantative detection of sample analyte. The reaction zone is contained in a test head, and the absorbent reservoir is concentrically positioned with respect to the test head, allowing telescoping movement of the absorbent reservoir with respect to the test head. The test head includes at least one control region and at least one reaction zone, with dividers isolating each control region and each reaction zone. Also, the test head comprises an aqueous impermeable face plate having at least one opening to allow liquid to reach the reaction zone, and a second opening to allow liquid to read the control zone. The test device is accompanied by a reagent pack sized and configured to supply a plurality of reagents to the reaction zone. To detect the detectable reaction product, the device includes a developer selected to participate in generating a colored substance from a chromophore.

In a third aspect, the invention features a method for detecting an analyte in a sample by reacting the analyte with at least one liquid reagent to form a detectable reaction product. The sample is contacted with the reaction zone, thereby causing the predetermined limited sample volume to flow through the means defining the reaction zone

Preferably, while the absorbent reservoir is retained in the first position, the sample is contacted with the reaction zone, thereby flowing the predetermined limited sample volume through the means defining the reaction zone; thereafter, the absorbent reservoir is moved in contact with the control absorbent; while the absorbent reservoir is maintained in contact with the control absorbent, the reaction zone is contacted with at least one of the liquid reagents, which flows through the reaction zone into the absorbent

reservoir. While the absorbent reservoir is maintained in the second position, the reaction zone may be contacted with one or more additional reagents which are allowed to flow through the reaction zone into the absorbent reservoir. A predetermined time is allowed after the sample absorption and before moving the absorbent reservoir into the second position, to allow the analyte to react with reactant in the reaction zone. After the detectable product is formed, the absorbent reservoir may be returned to the first position to enable detection of the reaction product while the absorbent reservoir is in the first position, i.e., in the absence of continued flow through the reaction zone caused by the absorbent reservoir. The absorbent reservoir can be maintained in the first position for a predetermined length of time, selected to control the desired reaction, so that both the volume of sample contacted with the reaction zone and the duration of that contact are controlled. The test head of the device is at one end and is positioned downwardly to be immersed in the sample and the liquid reagent(s). Then, after the detectable product is formed, the device is inverted to position the test head on top during detection of the product.

The invention involves a particularly rapid assay that is easy to use with a minimal sample volume. The test result is reliably read, with enhanced background contrast and with reliable controls. The test device is readily assembled with protection against error during assembly and use. The overall assay generally represents a significant cost savings. Since the size of the reaction zone is minimized, and flow is not wasted on regions outside the zone, the amount of reagent used is minimized. The number of steps involved

is reduced, thus reducing the opportunity for operator error. Mixing and measuring of reagents is avoided. Internal, self-executing controls provide increased reliability.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiment thereof, from the figures, and from the claims.

Description of the Preferred Embodiment

Drawings

Fig. 1 is a view of an immunoassay kit;

Fig. 2 is an exploded view of the dipstick of Fig. 1;

Fig. 2a is a view of the cap and barrel of the dipstick of Fig. 2, with other parts of the dipstick omitted.

Fig. 3 is a view, with parts broken away, showing assembly of the dipstick of Fig. 2;

Fig. 4 is a side view of the dipstick of Fig. 2. in section, with parts omitted for clarity, taken along 4-4 of Fig. 1;

Fig. 5 is an exploded cross-section of the dipstick of Fig. 2 taken along 5-5 of Fig. 2a, with parts omitted for clarity;

Fig. 6 is a view along 6-6 of Fig. 4;

Figs. 7A and 7B show, respectively, the two operating positions of the dipstick of Fig. 2;

Fig. 8 shows, in highly diagrammatic fashion, five steps in an assay using the kit of Fig. 1;

Fig. 9 depicts a key for reading the results of the assay of Fig. 8.

Structure

In Fig. 1 an immunoassay kit 10 includes a dipstick 12 and a reagent tray 14 which has a clear lid

16. There are four wells 18a-18d in tray 14, described in greater detail below. Tray 14 also includes an elongated slot 15 to accomodate dipstick 12 and a circular sample well 19 to receive the sample.

In Figs. 2-6, dipstick 12 includes a test head 20, a barrel 24, and a cap 40. Test head 20 (best shown in Fig. 6) is sized to be immersed in sample well 19 and in wells 18a-18d. Test head 20 has three liquid-receiving openings in face 22. Round openings 21a and 21b are positive and negative control openings, respectively, as explained below. Opening 23 is the analyte detection opening. An index notch 25 is positioned opposite positive control opening 21a to indicate the proper test head orientation when reading test results.

In Figs. 2-5, barrel 24 of the dipstick includes three axially extending fins 35 evenly spaced around the circumference of the interior of the barrel and integral with the barrel. As shown in Figs. 4 and 5, fins 35 extend to the head region of the dipstick, where they terminate in radial fins 26, which are shown in more detail in Figs. 2 and 5. Fins 26 are shaped and positioned to fit within three radial recesses 28 in the primary (or control) absorbent 30, described below, and each terminates in a sharp edge 27, designed to pierce depth matrix 32 during assembly.

A cylindrical absorbent reservoir 38, positioned within barrel 24, is described in greater detail below. Axial ribs 35 center the absorbent reservoir. Barrel 24 also includes an indexing block 52 extending radially beyond the circumference of the barrel.

Depth matrix 32 can be a glass fiber membrane (e.g., Gelman A/E; Pall 0-10) which is capable of absorbing microparticles as described below.

Control absorbent 30 is hydrophilic polyethylene material, which has slots 28 molded in it. For each test, a desired optimum sample volume is determined, and the control absorbent depth and porosity are selected accordingly. A typical volume of sample desired to be moved through the reaction zone is less than 400 µl and certainly less than 1 ml (most preferably less than 150-200 µl). A suitable control absorbent for a volume of 100-150 µl is a porous polyethylene (average pore size 40 µ) with a diameter of 0.410 inches and a thickness of 0.100 inches. Such material is available from Porex Technology, Fairburn GA or Chromex Inc., Brooklyn, New York.

The absorbent reservoir 38 comprises drawn cellulose acetate fibers of 3.5 to 4.5 denure. The fibers may be oriented parallel to the barrel axis (along the direction of fluid movement) to provide fast wicking and thereby reduce the total assay time. The fibers are treated with a plasticizer such as triacten to stiffen them and improve flow. The absorbent reservoir should have excess capacity for the total liquid volume to be moved through the reaction zone, e.g., at least about 5 ml.

Cap 40 includes cross-members 46 and offset ribs 43 (Fig. 5) to pinch and retain absorbent reservoir 38, as shown in Fig. 4. Indexing void 50 on the interior of cap 40 mates with indexing block 52 on the exterior of barrel 24. A ridge 47 extends around the circumference of the interior of cap 40 and mates with circumferential ridges 44 and 54 on the exterior of barrel 24.

Assembly

Solid portions of the dipstick are suitable plastic such as injection molded polypropylene. The barrel and test head are molded as separate parts.

Reservoir 38 is pinched between cross-members 46 and ribs 43, so that the reservoir is retained away from control absorbent 30. Cap 40 is forced over the end of barrel 24, with indexing void 50 positioned over indexing block 52. Ridge 47 is snapped over ridge 44, but not over ridge 54, so absorbent reservoir 38 is maintained apart from control absorbent 30 as shown in position A of Fig. 7.

After assembly, the microparticles are spotted through test head ports into the assay region. Specifically, latex microparticles with anti-analyte antibody can be spotted through analyte detection port 23 onto depth matrix 32. Latex particles containing the chromagen label (e.g., the enzyme) are spotted through positive control 21a. Particles non-reactive to the sample or the reagents are spotted through negative control 21b. The latex microparticles can be made of polystyrene. Proteins are immobilized on the particles by known techniques. See, e.g., Bangs, Uniform Latex Particles, Seragen Diagnostics. The particles are suspended in a buffer (e.g., 0.5%-1.0% weight loading in a standard saline buffer appropriate for the enzyme at issue) to be dropped onto the reaction zone.

Use

The user receives kit 10 with dipstick 12 nested in slot 15, and lid 16 covering tray 14. After removing the lid, sample is added to well 19 according to a protocol that will depend upon the sample and the precise nature of the assay.

Dipstick 12 is removed from the slot. At this stage, the cap 40 is retracted, and the absorbent reservoir 38 is spaced apart from the control absorbent (position A in Fig. 7).

The test head 20 is immersed in sample well 19 (Fig. 8, step 1). The amount of sample drawn through the reaction zone 32 is determined by control absorbent 30. Specifically, the porosity and dimensions of the control absorbent control the sample volume drawn into the reaction zone. The capacity of control absorbent 30 is limited--i.e., the control absorbent will not absorb all of the sample in well 19. Rather, once absorbent 30 is filled to capacity, sample flow through the reaction zone (i.e., from the receiving face of the zone to the outlet face of the zone) ceases. In that way, the capacity of control absorbent 30 meters the extent of flow of sample through the reaction zone predetermined (experimentally) to provide reliable analyte detection. That flow will depend on the amount of reagent available in the reaction zone, the intensity of the parameter being detected, the level of analyte as to which discrimination is desired, sample viscosity, and the sample volume available.

Once the dipstick has absorbed the desired sample volume (e.g. 10 seconds) (Fig. 8, step 2), the test head is removed from the sample well (Fig. 8, step 3). The user presses the cap inwardly (in the direction of the test head). As ridge 47 engages ridge 54, an audible click is produced. In this position (position B in Fig. 7), the absorbent reservoir 38 is forced in contact with the control absorbent (Fig. 8, step 4), so that liquid is wicked away from assay region 32 and control absorbent 30, into the reservoir 38.

The dipstick is then inserted serially into reagent wells 18a through 18d (Fig. 8, step 5). Each well is sized to hold the dipstick upright, by friction fit. Control over the amount of each reagent to be supplied to the assay region can be achieved in either

of two ways: 1) by controlling the time of immersion in each well; or 2) by limiting the amount of reagent in the well and ensuring immersion long enough to absorb the entire contents of the well.

The color development takes place in the depth matrix, because the microspheres are trapped in the depth matrix and the colored product remains in the microsphere.

The results are read as shown in Fig. 9. A sample enzyme-linked immunosorbent assay is described below by way of example and not as a limitation on the scope of the invention.

Example

Anti-viral antibody, conjugated to latex microparticles by standard techniques are spotted through analyte detection port 23 onto the depth matrix 32. Similar latex microparticles containing enzyme are spotted through positive control port 21a onto assay region 32. Paticle; are spotted through negative control port 21b.

Reagent wells are filled as follows:

18a: antibody-enzyme conjugate (30 sec. - 2 minutes.)

18b: wash solution (60 sec.)

18c: enzyme substrate (30 - 60 sec)

18d: stop solution -- A solution which either stops enzyme activity, and/or precipitates the substrate by changes in pH, ionic strength, or the addition of an inhibitor.

Suitable systems include well known alkaline phosphatase systems and horseradish peroxidas H_2O_2 systems.

Each well 18a-18d contains a slight excess of the reagent in question, absorbed within sponge matrices

56a-56d which release a reagent when compacted by the test head. In this way, reagent loss through evaporation in shipping and storage is avoided.

Other Embodiments

Other embodiments are within the following claims. For example, in the test head can be a separate piece from the barrel. The control absorbent and depth matrix can be manufactured as a single element. For example, an absorbent porous polyethylene substrate can be constructed to have constricted pores on the top surface using a cellulosic material.

The test tray can be packaged in a shrink-wrap plastic film, omitting the cover. The test tray can be modified by changing the spacing and location of the wells. For example, sample well 19 can be moved to one end of the dipstick slot 15 and wells 18a-18d can be spaced apart, with a groove included between each well to contain liquid that drips and avoid contaminating the wells.

A range of immunoassay techniques are performed with the device, including radioactive and fluorescent techniques as well as the colorimetric technique described in this patent application. The size of the device can be adjusted depending on assay volume. A pre-filter can be used to remove undesired elements of the sample that hinder the assay, e.g. red blood cells in a whole blood assay.

The number of openings or ports in the test head can be increased, e.g., multiple assays can be performed on the same sample, by spotting different analyte binding agents in different openings.

The test can be read by automated reading apparatus, as well as by visual inspection.

Claims

1. A test device for detecting an analyte in a liquid sample by treating the analyte with at least one liquid reagent to form a detectable reaction product, said test device comprising:

a) means defining an aqueous permeable, aqueous insoluble reaction zone, adapted to retain said detectable reaction product, said means defining a reaction zone comprising a first region exposed to receive said liquid sample and a second region, spaced from the first region; and

b) a control absorbent in liquid-transferring relation with said second region of said means defining a reaction zone, said control absorbent having a predetermined, limited, standard, liquid-absorbing capacity, said control absorbent and said means defining a reaction zone being positioned in said device in the manner that the control absorbent, in being filled to capacity, effectively meters a predetermined limited flow between said first region and said second region, said predetermined, limited, standard volume being selected to enable reliable detection of said analyte.

2. The test device of claim 1 further comprising:

c) an absorbent reservoir;

d) means retaining said absorbent reservoir in a first position spaced apart from liquid-transferring contact with said control absorbent; and

e) means moving said absorbent reservoir from said first position to a second position, in liquid-transferring contact with said control absorbent;

whereby said control absorbent controls the sample volume absorbed into said means defining a

reaction zone while said absorbent reservoir is in said first position; thereafter said absorbent reservoir is moved into said second position, and said means defining a reaction zone is contacted with at least one liquid reagent which is drawn into said absorbent reservoir.

3. The test device of claim 1 further comprising:

c) an absorbent reservoir;

d) means retaining said absorbent reservoir in a first position above and spaced apart from liquid-transferring contact with said control absorbent; and

e) means for moving said absorbent reservoir from said first position to be retained in a second position in liquid-transferring contact with the means defining a reaction zone.

4. A test device for detecting an analyte in a liquid sample by treating the analyte with at least one liquid reagent to form a detectable reaction product, said test device comprising:

a) means defining an aqueous permeable, aqueous insoluble reaction zone, adapted to retain said detectable reaction product;

b) an absorbent reservoir;

c) means retaining said absorbent reservoir in a first position above, and spaced apart from liquid-transferring contact with, said means defining a reaction zone; and

d) means for moving said absorbent reservoir from said first position to be retained in a second position in liquid transferring contact with the means defining a reaction zone;

whereby said sample is introduced to said means defining a reaction zone while said absorbent reservoir is in said first position, said absorbent reservoir is moved into said second position, a plurality of said liquid reagents are contacted separately with said means defining a reaction zone and are drawn upwardly into said absorbent reservoir.

5. A test device for detecting an analyte in a liquid sample by treating the analyte with at least one liquid reagent to form a detectable reaction product, said test device comprising:

a) means defining an aqueous permeable, aqueous insoluble reaction zone, adapted to retain said detectable reaction product;

b) a control absorbent in liquid-transferring relation with said second region of said means defining a reaction zone, said control absorbent having a predetermined, limited, standard, liquid-absorbing capacity, said control absorbent and said means defining a reaction zone being positioned in said device in the manner that the control absorbent, in being filled to capacity, effectively meters a predetermined limited flow between said first region and said second region, said predetermined, limited, standard volume being selected to enable reliable detection of said analyte.

c) an absorbent reservoir;

d) means retaining said absorbent reservoir in a first position above and spaced apart from liquid-transferring contact with said control absorbent;

e) means for moving said absorbent from said first position to be retained in a second position in liquid-transferring contact with the means defining a reaction zone; and

f) latch means to restrain movement of said absorbent reservoir from said second position to said first position;

whereby said sample is introduced to said means defining a reaction zone while said absorbent reservoir is in said first position, said absorbent reservoir is moved into said second position, and a pluarlity of said liquid reagents are contacted separately with said means defining a reaction zone and are drawn upwardly into said absorbent reservoir.

6. The device of claim 1 or claim 4 wherein said means defining a reaction zone comprises at least one reactant participating in a reaction to form said detectable product.

7. The device of claim 1 or claim 4 wherein said means defining a reaction zone is impermeable to said analyte.

8. The device of claim 1 or claim 4 wherein said first region of said means defining a reaction zone and said second region thereof are opposite surface of a flat member.

9. The device of claim 1 or claim 4 wherein said reaction product is detected by visual inspection, and said means defining a reaction zone is visible by external inspection.

10. The device of claim 1 or claim 4 wherein said reaction product is detected by determining a characteristic of said reaction zone selected from the group consisting of color intensity, optical density, reflectance density, pH, fluorescence, and conductivity.

11. The device of claim 10 further comprising a means defining a contrast region surrounding said reaction zone to aid in determining said reaction zone characteristic by contrasting with said reaction zone in respect to said characteristic.

12. The device of claim 10 further comprising an intensity scale for quantative detection of sample analyte.

13. The test device of claim 2 or claim 4 wherein said means defining a reaction zone is contained in a test head, and said absorbent reservoir is concentrically positioned with respect to said test head, allowing telescoping movement of said absorbent reservoir with respect to said test head.

14. The test device of claim 3 or claim 4 wherein said device comprises a latch means to restrain movement of said absorbent reservoir from said first position to said second position, whereby a plurality of said liquid reagents can be introduced to said reaction zone while said absorbent reservoir is latched in said second position.

15. The test device of claim 14 wherein said device comprises means to release said latch after said detectable product is formed, whereby said absorbent reservoir is returned to said first position while the reaction product is detected.

16. The test device of claim 1 or claim 4 wherein said means defining a reaction zone is integral with said control absorbent.

17. The test device of claim 1 or claim 4 wherein said means defining a reaction zone is attached to a face of said control absorbent.

18. The test device of claim 1 or claim 4 wherein said means defining a reaction zone defines at least one reaction region and at least one control region, and said device comprises means for isolating each control region from and each reaction zone.

19. The test device of claim 18 wherein said means defining a reaction zone and control region comprises an aqueous impermeable face plate having at least one opening to allow liquid to reach each reaction zone, and at least one opening to allow liquid to reach each said control region.

20. The test device of claim 1 or claim 4 further comprising a reagent pack sized and configured to supply a plurality of reagents to said reaction zone.

21. The test device of claim 1 or claim 4 wherein said means defining a reaction zone comprises a specific binding partner for said analyte.

22. The test device of claim 1 or claim 4 wherein said liquid reagents comprise means for generating a detectable reaction product.

23. A method for detecting an analyte in a sample by reacting the analyte with at least one liquid reagent to form a detectable reaction product, said method comprising:

a) a control absorbent in liquid-transferring relation with said second region of said means defining a reaction zone, said control absorbent having a predetermined, limited, standard, liquid-absorbing capacity, said control absorbent and said means defining a reaction zone being positioned in said device in the manner that the control absorbent, in being filled to capacity, effectively meters a predetermined limited flow between said first region and said second region, said predetermined, limited, standard volume being selected to enable reliable detection of said analyte;

b) contacting said sample with said means defining a reaction zone and thereby allowing said predetermined sample volume to flow through said means defining a reaction zone;

c) allowing formation of said detectable reaction product; and

d) detecting said reaction product.

24. The method of claim 23 wherein said device comprises an absorbent reservoir; means retaining said absorbent reservoir in a first position spaced apart from liquid-transferring contact with said control absorbent; and means moving said absorbent reservoir from said first position to a second position, in liquid-transferring contact with said control absorbent; and said method comprises the steps of:

a) contacting said sample with said means defining a reaction zone while said absorbent reservoir is in said first position, and thereby flowing said predetermined, limited flow through said means defining a reaction zone;.

b) thereafter, moving the absorbent reservoir in contact with the control absorbent; and

c) while said absorbent reservoir is maintained in contact with said control absorbent, contacting said reaction zone with at least one of said liquid reagents and allowing said reagent to flow through said reaction zone into said absorbent reservoir.

25. The method of claim 24 wherein said method further comprises:

contacting said reaction zone with a second one of said liquid reagents while maintaining said absorbent reservoir in contact with said control absorbent, and allowing said second liquid reagent to flow through said reaction zone into said absorbent reservoir.

26. The method of claim 24 wherein one of said liquid reagents is provided in a predetermined quantity and step c) of claim 21 is performed until said substantially all of said quantity is flowed through said reaction zone.

27. The method of claim 24 wherein an excess of one of said liquid reagents is provided, and step c) of claim 21 is performed for a predetermined time.

28. The method of claim 24 wherein said means defining a reaction zone comprises at least one reactant confined therein, and said method further comprises waiting a predetermined time after step a) of claim 21 and before step b) of claim 21, in order to allow said analyte to react with said reactant.

29. The method of claim 24 wherein said detectable product is formed, and thereafter said absorbent reservoir is returned to said first position

to enable detection of said reaction product while said absorbent reservoir is in said first position, in the absence of continued liquid flow through the reaction zone caused by the presence of the absorbent reservoir in the first position.

30. The method of claim 24 wherein the absorbent reservoir is maintained in said first position for a predetermined length of time selected to control the desired reaction, whereby both the volume of sample contacted with the reaction zone and the duration of contact of the sample and reaction zone are controlled.

31. The method of claim 24 wherein said device comprises a test head at one end thereof comprising said means defining a reaction zone, said method comprising:

a) immersing said test head in the sample and in said liquid reagents with the device oriented downward; and

b) after said detectable reaction product is formed, inverting said device to position said test head at the top thereof during detection of the product.

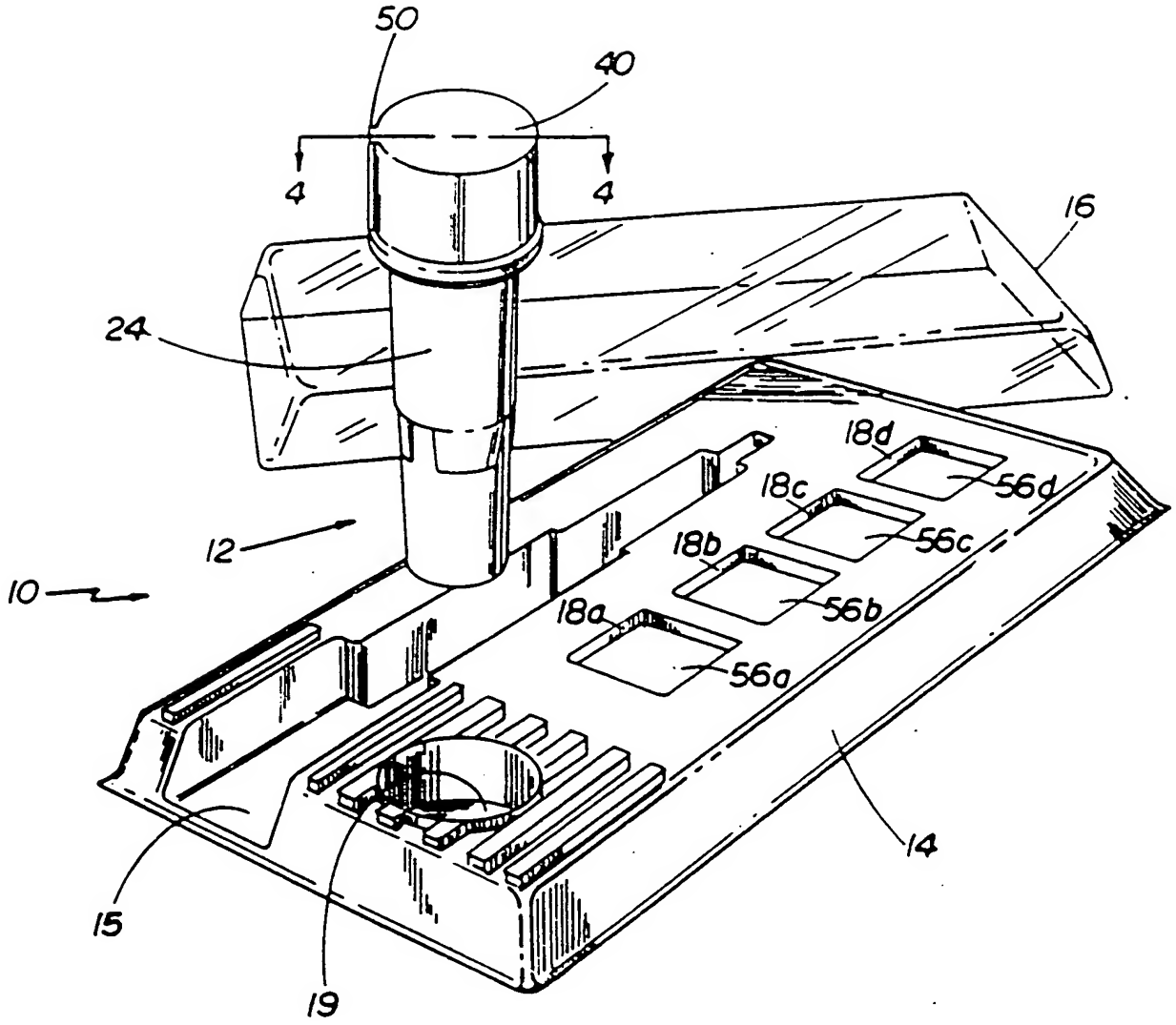


FIG. 1

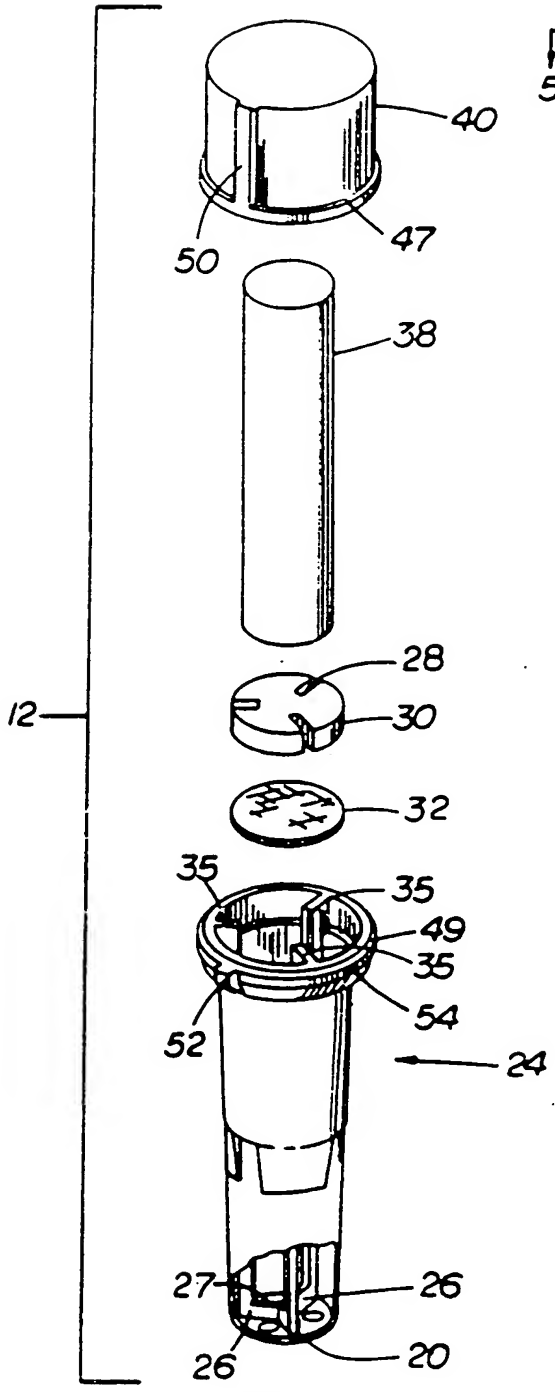


FIG. 2

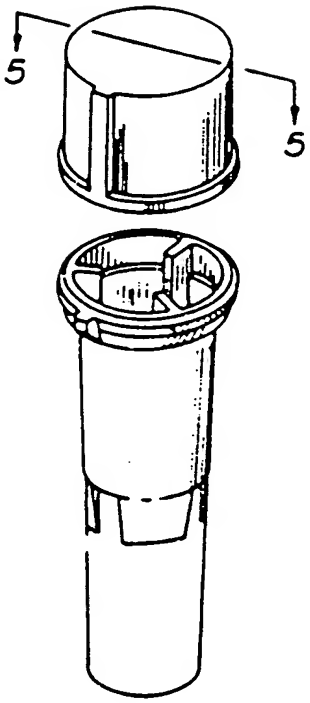
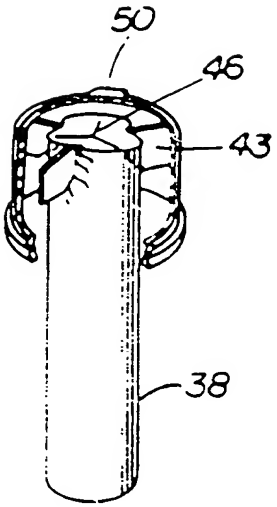


FIG. 2a



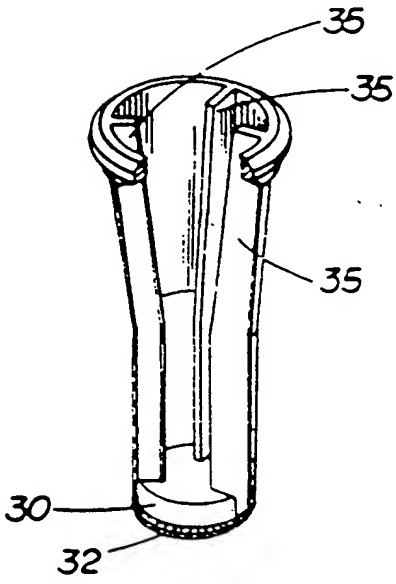


FIG. 3

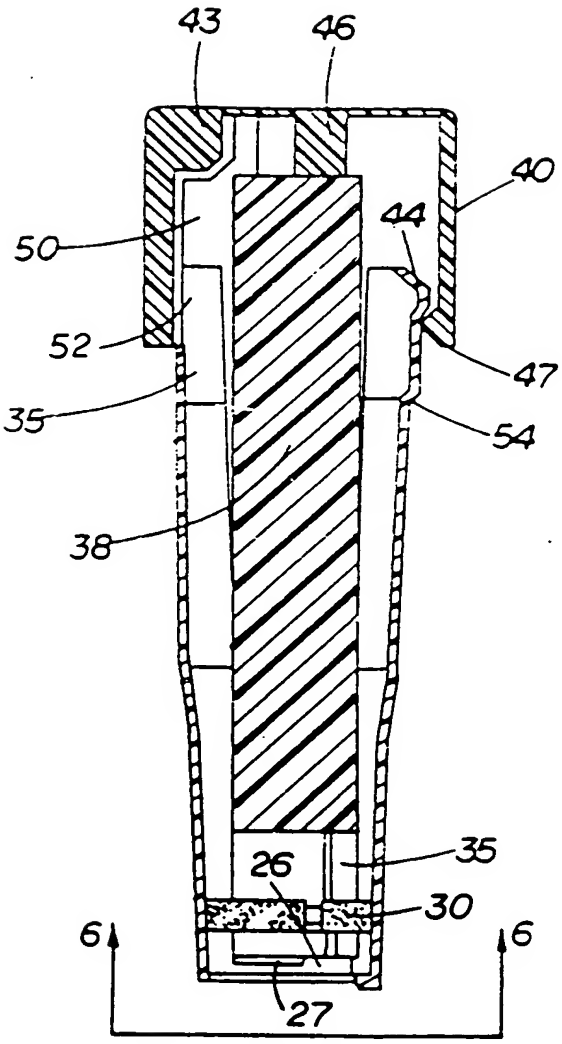


FIG. 4

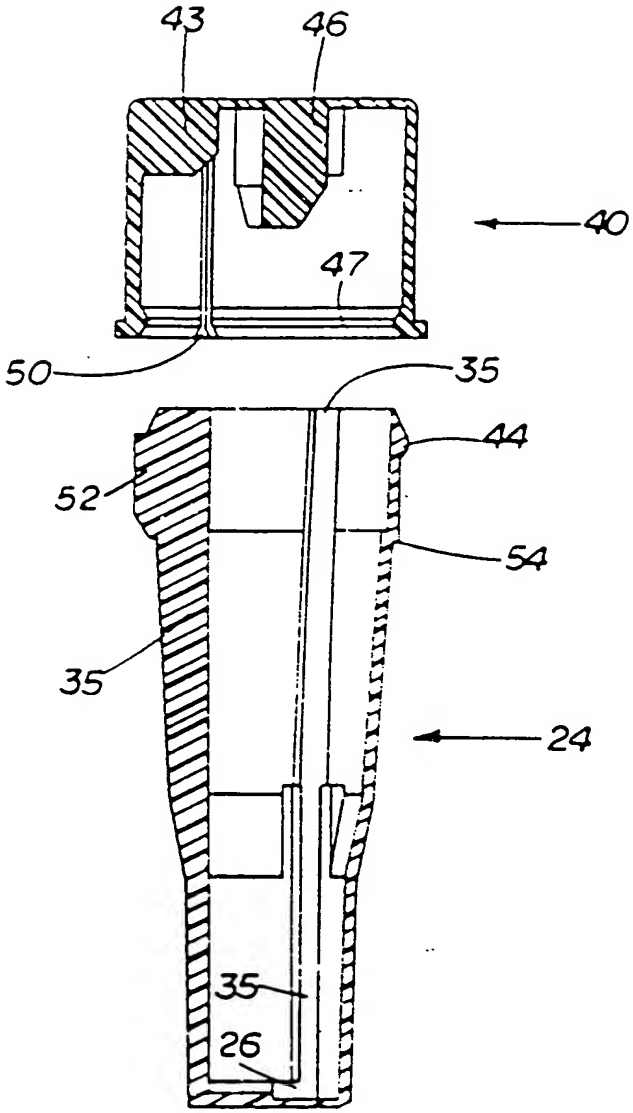


FIG. 5

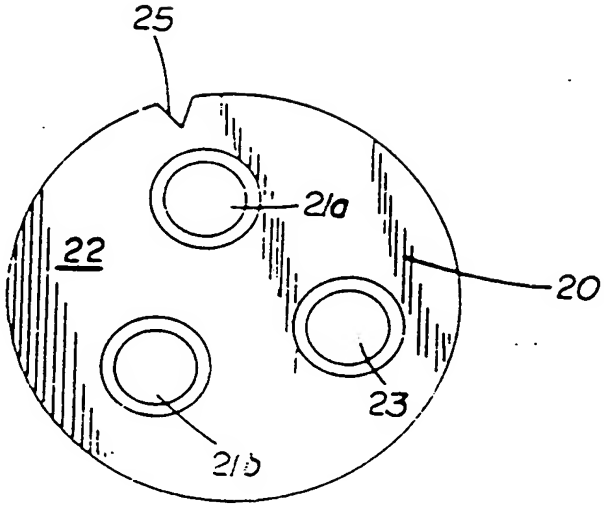
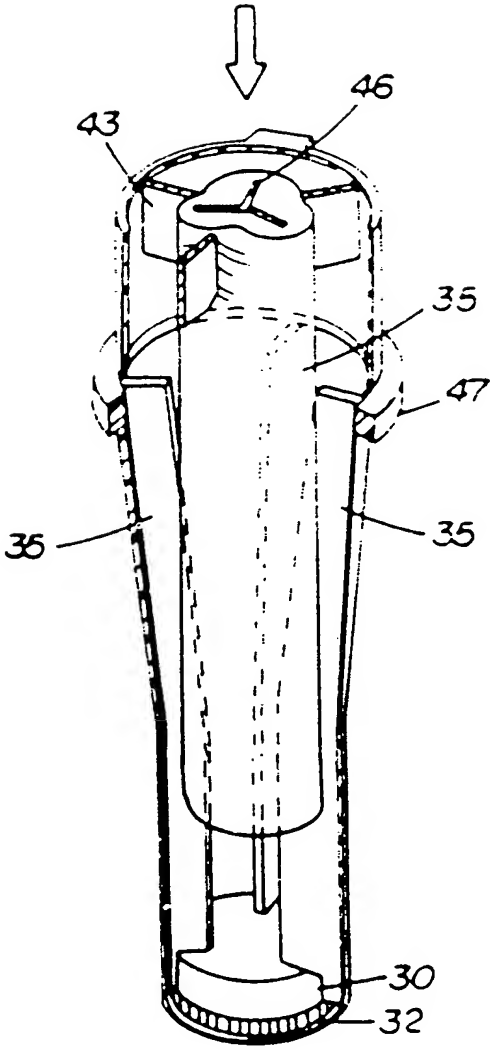
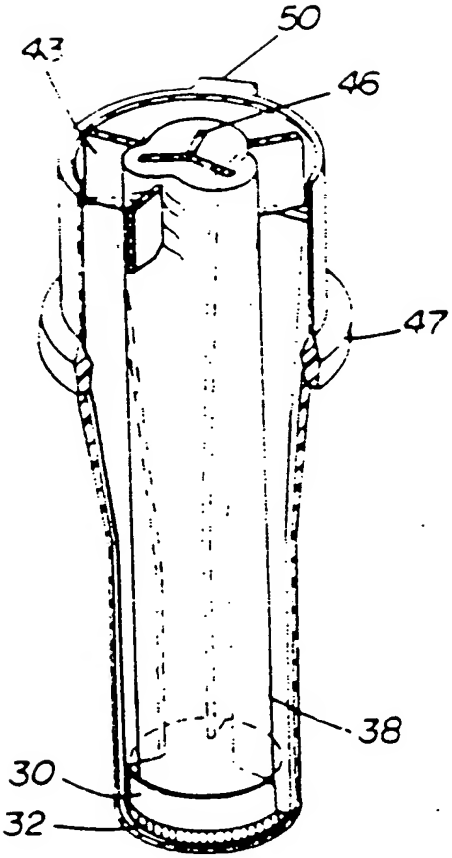


FIG. 6



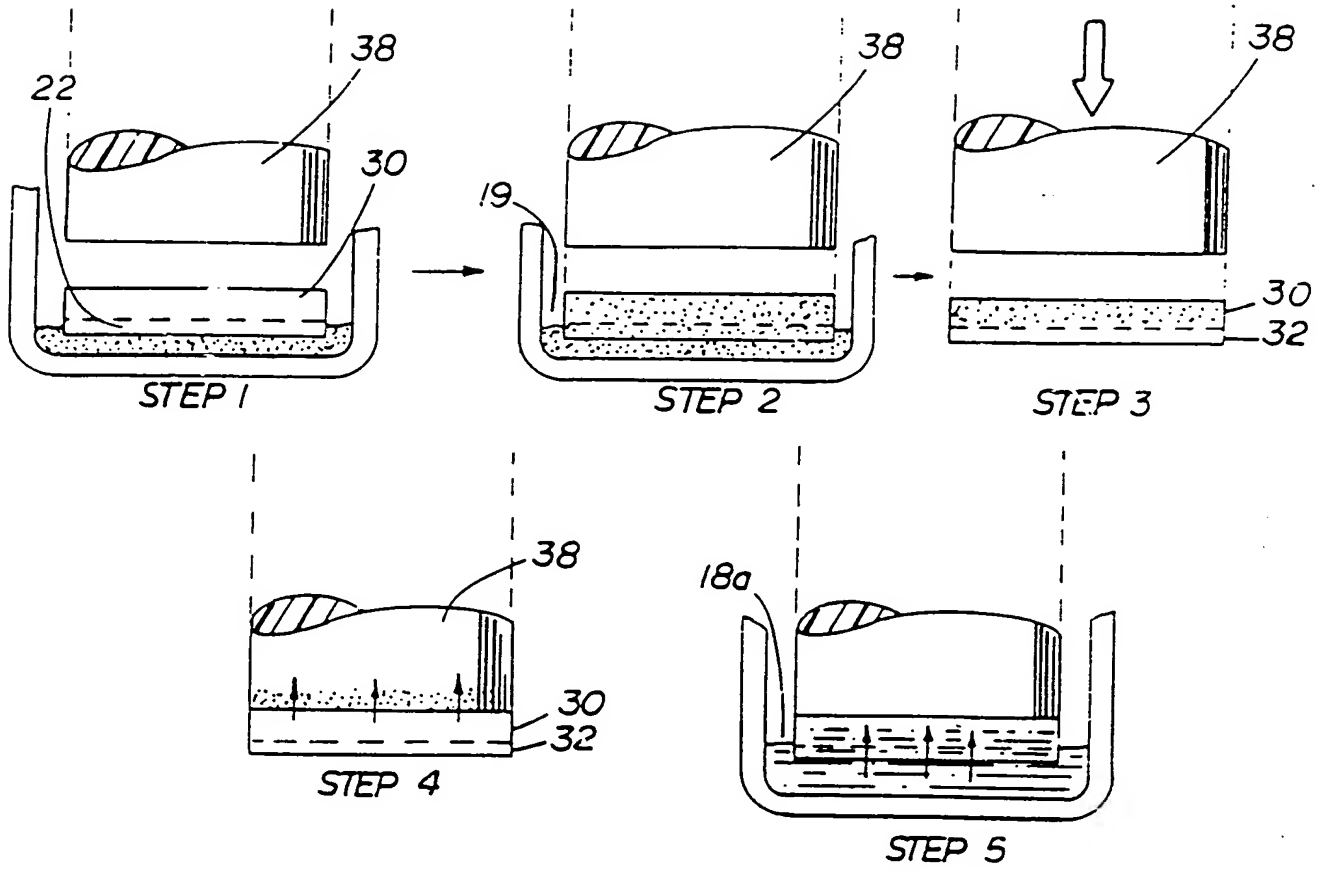
POSITION A

FIG. 7a



POSITION B

FIG. 7b


38
22
30
STEP 1
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19
STEP 2
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30
32
STEP 3
38
30
32
STEP 4
18a
STEP 5

FIG. 8

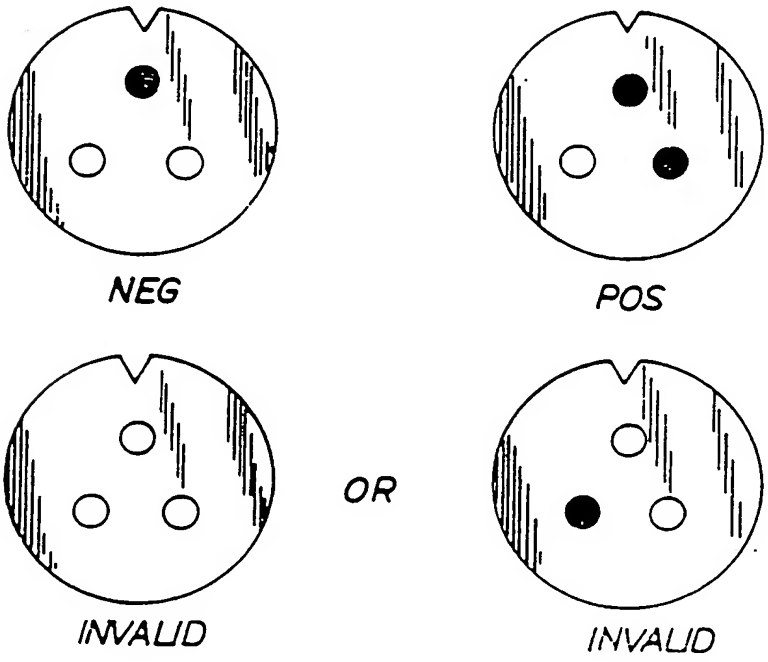
NEG
POS
OR
INVALID
INVALID

FIG. 9

INTERNATIONAL SEARCH REPORT

International Application No PCT/US89/01788

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ³

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4): G01N 21/77, 21/73, 33/543 US.CL.: 422/56,58,60,61
US.CL.: 435/805; 436/518,535,541,170,810,824

II. FIELDS SEARCHED

Minimum Documentation Searched ⁴

Classification System ⁵	Classification Symbols
U.S.	422/55,56,57,58,59,60,61,68,69; 435/7,805; 436/514,518,535,541,164,169,170,172,149,163, 805,806,807,810,824,825

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁶

III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴

Category ⁷	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
X Y	US, A, 3,883,629 (BAGSHAWE) 10 June 1975, see the entire document.	1-10,13, 16-17,21-23 5,11-12, 14-15,18-20, 24-31
X Y	US, A, 4,246,339 (COLE et al) 20 January 1981, see the entire document.	4,6-10, 13 18-19,21-22 1-3,5-31
X Y	US, A, 4,366,241 (TOM et al) 28 December 1982, see the entire document.	1,6-10,16 21-23 2-22,24-31
X Y	US, A, 4,632,901 (VALKIRS et al) 30 December 1986, see the entire document.	1,6-11,16 18-19,21-23 2-22,24-31
X Y	EP, A, 0,253,579 (PETRO et al) 20 January 1988, see the entire document.	1,6-10,16 21-23 2-22,24-31

* Special categories of cited documents: ¹⁵

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search ³	Date of Mailing of this International Search Report ³
27 April 1989	26 JUN 1989
International Searching Authority ¹	**Signature of Authorized Officer ¹⁰**
ISA/US	Robert J. Hill, Jr.